PATENT

Case Docket No.: NOVCEL.3CPDDDVC
Filing Date: January 20, 2004

Applicant(s)	:	**Hubbell, et al.**
App. No.	:	**10/761,180**
Filed	:	**January 20, 2004**
For	:	**Gels For Encapsulation Of Biological Materials**
Examiner	:	**Berman, Susan W.**
Group Art Unit	:	**1711**

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

March 1, 2006
(Date)

Kirk Hahn, Reg. No. 51,763

DECLARATION UNDER 37 C.F.R. § 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, JEFFREY A. HUBBELL, declare as follows:

2. My address is Rue Louis de Savoie 27, CH-1110 Morges, Switzerland.

3. I am a co-inventor of the invention in U.S. Application Serial No. **10/761,180** entitled "**Gels For Encapsulation Of Biological Materials**" (the "Application"); and U.S. Application Serial No. 07/843,485 ("Priority Document I"), now abandoned, filed February 28,1992 and U.S. Application Serial No. 07/870,540 ("Priority Document II"), filed April 20, 1992, now abandoned, on which this application claims priority.

4. I understand that the Examiner has cited Soon-Shiong et al., U.S. Patent Numbers 5,700,848 ("Soon-Shiong '848"), 5,705,270 and 5,846,530 with effective filing dates of October 29, 1991 in her rejection of certain claims presented in the present Application.

5. I understand that the Examiner has cited Soon-Shiong et al., U.S. Pat. No. 5,545,423 ("Soon-Shiong '423"), 5,759,578, 5,788,988 and 5,879,709 with effective filing dates of November 25, 1991 in her rejection of certain claims presented in the present Application.

6. In 1991, I was a Professor at the University of Texas at Austin with a research laboratory investigating hydrogels. The inventions described and claimed in the Application, Priority Document I, and Priority Document II were discovered, conceived and reduced to practice in my research laboratory.

7. Prior to October 29, 1991, I, along with my co-inventors, reduced to practice in the United States the method of encapsulating biological materials, which comprises mixing the biological material with an alginate, forming microcapsules, coating the microcapsules with a photoinitiator, mixing the microcapsules containing the biological material in an aqueous macromer solution, polymerizing the gel using a light source and generating macrocapsules in accordance with the subject matter claimed in this application.

8. Attached hereto is a copy of a letter ("Exhibit A") sent by me before October 29, 1991 describing the invention. Exhibit A is a true and correct copy of the original document wherein it has been masked to remove dates and nonpertinent business discussions.

9. Exhibit A discloses the "PEG gel method for encapsulation" which "allows us to build up a layer of PEG gel around an algin-PLL-algin capsule" and it improves biocompatibility, strength, and stability of the macrocapsule.

10. Attached hereto is a copy of an Intellectual Property Disclosure ("Exhibit B") of the University of Texas at Austin written by me and signed by me, Chandrashekhar P. Pathak, and Amarpreet S. Sawhney, and dated prior to October 29, 1991. Except for the masked date, Exhibit B is a true and correct copy of the original document.

11. Exhibit B, page 2 describes a polyethylene glycol (PEG) macromer that is modified at both ends to incorporate an unsaturated, polymerizable moiety there. One preferred method is to modify PEG with acryloyl chloride, thus producing PEG diacrylate (PEG DA).

Following purification, the PEG-DA solution is mixed with a photosensitizer (ethyl eosin and triethanolamine) and the cells to be encapsulated. Small droplets are formed and passed through an argon ion laser beam (514 nm, green) to induce polymerization.

12. The terms and methods used in Exhibit B are supported in the Application, Priority Document I and Priority Document II, and can be seen to include the same steps as required by claim 149.

13. PEG of Exhibit B is a species of macromer (Application, page 21, lines 1-2; Priority Document I, page 26, lines 33-35; Priority Document II, page 18, lines 11-13) that is described as water soluble (Application, page 20, lines 27-28; Priority Document I, page 26, lines 32-35; Priority Document II, page 18, lines 21-25).

14. Exhibit B (page 9, lines 15-17) shows PEG dissolved in water, thus forming an aqueous macromer solution.

15. The Application describes eosin dye as the preferred photoinitiator (Application, page 13, line 23), and more particularly, ethyl eosin (Application, page 22, lines 8-9; Priority Document I, page 27, lines 12-13; Priority Document II, page 19, lines 4-14; Exhibit B, page 8, line 2) which is used in the method described in Exhibit B.

16. As further described in Exhibit B (page 3, lines 1-7), encapsulation of eukaryote and prokaryote cells are important applications of this technology.

17. Triethanolamine described in Exhibit B is an optional co-catalyst that stimulates the free-radical polymerization reaction (Exhibit B, page 2, lines 33-34; Application, page 13, line 15.)

18. The co-catalyst is described in Priority Document II at page 19, lines 14-23, and its optional inclusion in the method is described at page 20, lines 13-14 and in Priority Document I, page 27, at lines 11-12.

19. The Application describes the required radiation for polymerization as either visible or long wave ultraviolet light which is nontoxic to biological materials (Exhibit B, page 2, lines 34-35; Application, page 10, line 7 and page 11, lines 3-4; Priority Document I, page 34, lines 8-11 and lines 22-25; Priority Document II, page 12, lines 5-8 and lines 22-25).

20. Exhibit B (page 2, line 35), Application (page 13, line 19), Priority Document I, (page 27, lines 15-17), and Priority Document II (page 19, lines 23-24) further describe the most preferred wavelength as 365-514 nm and state that an appropriate source is an argon ion laser (Application, page 23, lines 1-2; Priority Document I, page 34, lines 31-34; Priority Document II, page 29, lines 3-5).

21. Thus, Exhibit B carries out the steps, where a cell is encapsulated by an aqueous macromer solution when the macromer is polymerized by a photoinitiator after exposure to light radiation. Exhibit B bears dates prior to October 29, 1991.

22. Accordingly, Exhibit B demonstrates that the invention as described in the Application was conceived and reduced to practice in the United States before the filing date of Soon-Shiong '848.

23. The disclosure of the encapsulation method "Microcapsule interfacial Polymerization Method" appears in the Application at page 16, line 1 through page 17, line 8.

24. This is the method cited in the Examiner's rejection as anticipated by Soon-Shiong '848. (See Application page 16, line 1, this method is also described in Priority Document I, page 22, lines 1-2 and page 31, "Example 7").

25. Attached here is a copy of a letter, forwarding three technical documents, ("Exhibit C") written by me before October 29, 1992. The titles on the disclosures were "PEO gels for the encapsulation of biological materials" ("Exhibit D"), "Visible laser polymerized polyethylene glycol) gel coatings for enhanced biocompatibility", and "Biodegradable, cell nonadhesive hydrogels from water soluble, photopolymerizable precursors". Exhibit C is a true and correct copy of the original document and has been masked to remove dates and nonpertinent business discussions.

26. Important features disclosed in Exhibit D; include "to treat microcapsule membranes containing living cells" (page 2, lines 9-10), "polymerization of a macromolecular precursor" (page 2, lines 10-11), and "fast reactions and visible light"(page 2, line 12).

27. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. Moreover, these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: 22 Feb 2006

Jeffrey A. Hubbell

EXHIBIT A

Jeffrey A. Hubbell, Ph.D.
3006 Beverly Road
Austin, Texas 78703
(512) 454-0264
FAX (512) 471-8799

[redacted]

Patrick Soon-Shiong
Diabetes Research and Management Center
Los Angeles, CA

By telefax: 213-553-1340

Dear Patrick:

I wanted to give you some food for thought before our discussion this Friday. We have been making very rapid progress on the PEG gel method for encapsulation. The technique is somewhat of a modification of that which you read about in the ADA would-be proposal. The modification allows us to build up a layer of PEG gel around an algin-PLL-algin capsule. In this way, the permselectivity (i.e. immunoprotectivity) of the capsule is exactly as it is in the normal capsule, but the biocompatibility is markedly improved. An addition benefit is that the capsule becomes stronger and likely more stable in that the outer layer is covalently bonded and should prevent the escape of PLL or algin. This is important, since breakage has been a problem.

[redacted]

My assessment of this technology is that before (with PLL-g-PEO) we have struck silver, but now we have struck gold. I am very optimistic that this material will take the dogs much beyond the 4-6 month duration that we are now observing. It is quite easy to practice, and adds about 25 minutes to the encapsulation process.

[redacted]

Sincerely yours,

Jeffrey A. Hubbell

EXHIBIT B

Hubbell

DEPARTMENT OF PHYSICS
THE UNIVERSITY OF TEXAS AT AUSTIN
Austin, Texas 78712-1081 · (512) 471-1152 · (512) 471-1153

EXECUTIVE VICE PRESIDENT AND PROVOST

[redacted]

CONFIDENTIAL

RECEIVED

[redacted]

MEMORANDUM

TO: Mr. Dudley Doble, Dr. Dale Klein, Mr. Wayne Kuenstler, Dr. Richard W. Miksad, Ms. Patricia Ohlendorf, Dr. Bob G. Sanders, Mr. Joe Powell, Dr. Eugene Wissler

FROM: Austin M. Gleeson

SUBJECT: *Laser Photopolymerized Gels for the Encapsulation of Cells*

Dr. Jeff Hubbell, Assistant Professor in the College of Engineering has submitted the attached invention disclosure. We will discuss this at our next meeting on [redacted] in RLM 5.210.

AMG:jd

Attachment

COLLEGE OF ENGINEERING
THE UNIVERSITY OF TEXAS AT AUSTIN
[redacted]
Department of Chemical Engineering • Austin, Texas 78712-1062 • (512) 471-5238 • FAX (512) 471-7060

MEMORANDUM

CONFIDENTIAL

TO: Austin Gleeson, Professor
Physics Department

FROM: Jeff Hubbell, Assistant Professor

SUBJECT: Invention Disclosure: Laser Photopolymerized Gels for the Encapsulation of Cells

DATE: [redacted]

Enclosed is a disclosure for consideration by your committee for potential action by the University. We have not disclosed the invention elsewhere and will delay disclosure until some action has been taken relating to patent protection. As such, I would appreciate your timely attention.

Thank your for your assistance in this matter.

cc: C.P. Pathak
A.S. Sawhney

INTELLECTUAL PROPERTY DISCLOSURE

The University of Texas at Austin

I. Descriptive

1. Title of Intellectual Property:

Laser photopolymerized gels for the encapsulation of cells.

2. Brief description. Is the invention a new process, composition of matter, a device, trade secret, technology, is it computer software, or one or more products? A new use for or an improvement of an existing product or process?

The intellectual property is:

(i) a process for synthesizing polymeric gels with controlled permeability,

(ii) polymeric gels that have been so synthesized, and

(iii) the application of such a process and material in the encapsulation of cells.

The most important application of encapsulated cells is the encapsulation of islets of Langerhans. The description will be primarily in the light of this example. If the encapsulation membrane possesses the appropriate permselectivity, then nonhuman islets can be transplanted into a human diabetic patient, without immunosuppression, to treat that patient's diabetes. The desired permselectivity is such that molecules smaller than about 70,000 g/mol are allowed to pass through the material, and molecules larger are not; in this way, nutrient, waste products, insulin, and selected transport proteins such as albumin are allowed access to the encapsulated cells, but antibodies and complement proteins are not, thus providing immunoprotection.

The requirements for successful encapsulation are (1) nontoxicity, (2) membrane stability, (3) permselectivity, and (4) biocompatibility. Previous approaches have lacked somewhat in membrane stability and greatly in biocompatibility.

The gels of the present invention allow the formation of immunoisolatory barriers around islets of Langerhans that perform at least as well as other approaches regarding characteristics 1 and 2, better in 3, and much better in 4.

The gel is produced as follows:

Prior to encapsulation, polyethylene glycol (PEG) of a given molecular weight (denoted by a number, in g/mol, following the abbreviation PEG, e.g. PEG 400 refers to PEG of molecular weight 400 g/mol) is modified at both ends to incorporate an unsaturated, polymerizable moiety there. Our preferred method is to modify PEG with acryloyl chloride, thus producing PEG diacrylate (PEG DA). Following purification, a solution of PEG DA is mixed with the cells to be encapsulated, along with a photosensitzer. Our preferred method is to use ethyl eosin with triethanolamine as the photosensitizer system. Small droplets are formed and passed through an argon ion laser beam (514 nm, green), whereupon they polymerize. The use of the photosensitizer with the visible light illumination avoids cellular damage that might be associated with ultraviolet illumination. The chemistry is outlined in the Appendix

Other biomedical applications for this encapsulation technology are the transplantation if encapsulated neurotransmitter-releasing cells in the brain for the treatment of Parkinson's disease, the transplantation of liver cells, and the transplantation of thyroid and parathyroid cells.

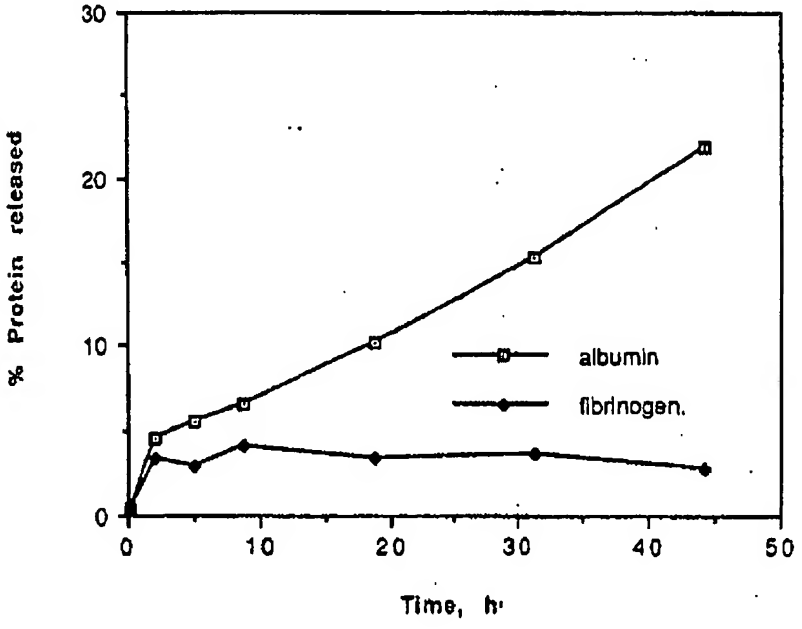
Applications in nonbiomedical areas of biotechnology are the encapsulation of cells, prokaryotic or eukaryotic, for fermentation. For these applications, however, it is likely that existing, simpler technologies would be just as effective.
Our supporting data are summarized below:

(1) Nontoxicity: Human fibroblasts were encapsulated as described in a 67% PEG 400 DA, 33% PEG 18000 DA gel. Samples were stained with trypan blue immediately after encapsulation, and at 1, 2, 6, and 24 hours. This dye stains dead cells. At all time points, less that 1% of encapsulated cells were stained, indicating viability and lack of toxicity.

(2) Stability: The gels are held together by covalent bonds that are not subject to biological degradation. Experimental confirmation of this is in progress.

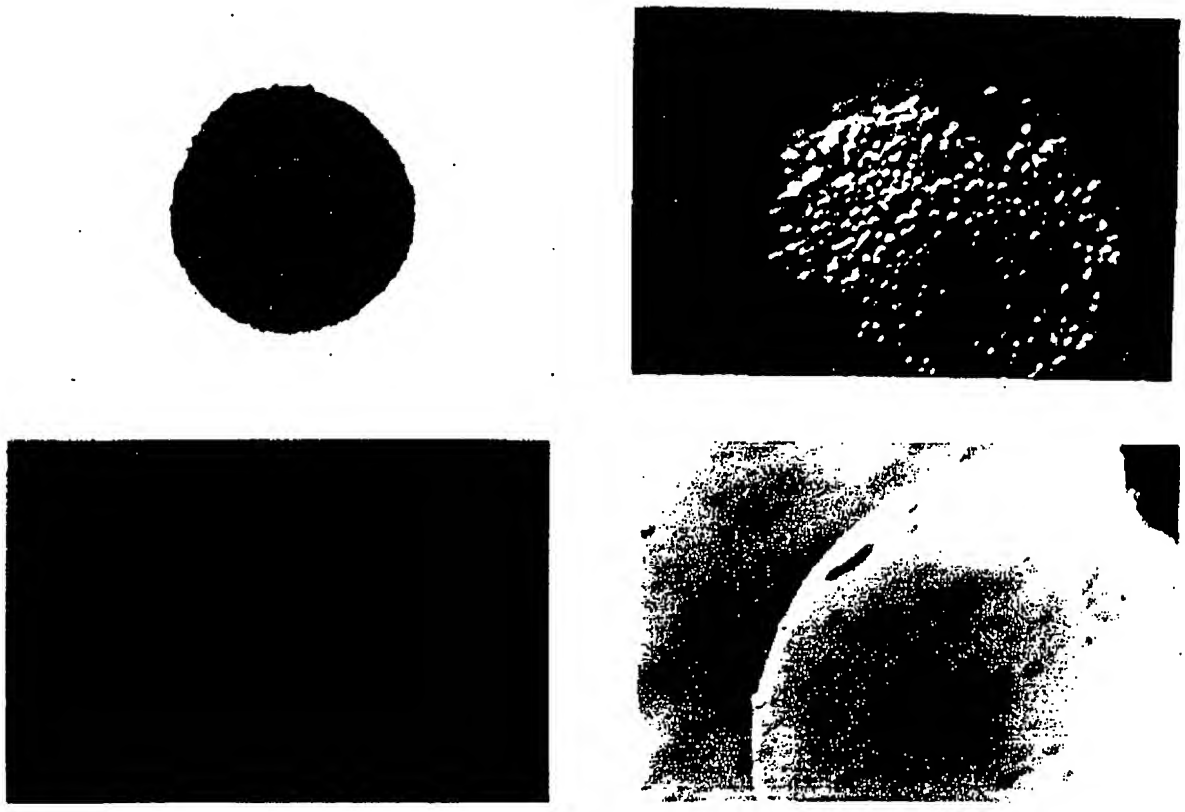
(3) Permselectivity. The ratio of low molecular weight PEG DA (such as 400 g/mol) to high (such as 5000 or 18500) can be used to control the permselectivity of the gels. This is illustrated by the following results. When albumin was encapsulated within a PEG 5000 DA gel, nearly all of it diffused out of the gel within 24 hours, using a total protein assay. When a gel was made from a 15% solution of 33% PEG 400 DA with 67% PEG 5000 DA, the rate of protein diffusion was markedly reduced, as shown in Fig. 1. Furthermore, the diffusion of proteins through the gel was dependent upon molecular weight: albumin (MW 64,500) diffused through the gel at a slow rate, and fibrinogen (MW about 400,000) did not diffuse through the gel, as shown in Fig. 1. The initial release of both albumin and fibrinogen from the gel is associated with the release of proteins at the surface of the gel; this initial burst does not represent diffusion of proteins out of the gel.

Figure 1. Diffusion of proteins from PEG gels.



(4) Biocompatibility: PEG 5000 DA gels and 65% PEG 5000 DA, 35% PEG 18000 DA gels (predicted to enhance biocompatibility) were implanted intraperitoneally in mice. The gel beads were explanted after 4 days and observed by phase contrast light microscopy. The PEG 5000 DA gel was observed to be covered with a monolayer of cells, the identity of which was not determined. The 65% PEG 5000 DA, 35% PEG 18000 DA beads were observed to be entirely free of attached cells. Fig. 2a and 2b show two representative views of PEG 5000 DA gel capsules recovered after 4 days, and Fig. 2c and 2d show two representative views of the 65% PEG 5000 DA, 35% PEG 18000 DA gel capsules after recovery.

Figure 2. Tissue response to PEG gels.



3. From the description, pick out and expand on novel and unusual features. How does the idea differ from present technology or software? What problems does it solve or what advantages does it possess?

The capsules made according to this invention appear to be extraordinarily biocompatible, and this feature has been notably lacking in previous approaches, preventing their success in treating diabetes. The present invention allows a unique combination of permselectivity with biocompatibility while allowing nontoxicity. The use of the laser in polymerization allows very short exposure times of cells to the reactive mixture, thereby reducing cytotoxicity.

4. If not indicated previously, what are possible uses for it? In addition to immediate applications, are there other uses that might be realized in the future?

These were described above.

5. Does the idea possess disadvantages or limitation? Can they be overcome? How?

No disadvantages are currently recognized for the primary application.

6. Sketches attached.

(i) Schematic of the process. Attached as an appendix.

(ii) Photographs of explanted microcapsules. Included in text above.

(iii) Figure showing controlled permselectivity. Included in the text above.

II. Other Pertinent Data

1. Are there publications --theses, reports, preprints, reprints, etc.-- pertaining to the idea? Please list with publication dates, and attach copies insofar as possible. Include manuscripts for publication (submitted or not), news releases, feature articles, and items from internal publications.

None.

2. Are laboratory records and data available? Give reference numbers and physical location, but do not enclose.

Records and data are in the possession of C.P. Pathak, CPE 4.436.

3. Are related patents, copyrights or other publication known to the inventor? Please list.

None on the use of photopolymerized PEG gels for cell encapsulation, on laser photopolymerized gels of any type for cell encapsulation, or on the control of permselectivity for PEG gels.

4. Date, place, and circumstances of first public disclosure.

The invention has not been disclosed and we will delay disclosure until a patent application has been filed.

5. Was the work that led to the development of the idea sponsored? If yes, attach a copy of the contract or agreement if possible, and fill in the appropriate blanks below:

a. Title of government agency: Texas Advanced Technology Program.

b. Name of industrial company: NA

c. Name of university sponsor: NA

d. Other sponsor: NA

6. Any commercial interest shown at this stage? Name companies and specific persons if possible.

Interest in the invention is very high. I have filed a patent, through UT, for another approach to biocompatible microencapsulation membranes, and I consider the PEG gel invention to be superior to this previous one. I am well-connected with the company that is leading in the area of islet transplantation, and I am positive that they would license the invention, as they did the previous one.

a. Do you know of other qualified firms? Please list.

Trancel Corp, Santa Ana, Califomi; Samuel D. Anderson, President.

7. Are there colleagues on campus who are already knowledgeable about the work and its potential impact? If so, please list two of them:

1. Adam Heller, Chemical Engineering, 471-8874 (Aware of some of the work and some potential impact.)

2. George Georgiou, Chemical Engineering, 471-6975 (Aware of some of the work and some potential impact.)

8. Names and titles of inventors:

a. Jeffrey A. Hubbell, Assistant Professor

b. Chandrashekhar P. Pathak, Postdoctoral Fellow

b. Amarpreet S. Sawhney, Graduate Research Assistant

For more information, contact Jeffrey A. Hubbell, 471-1690

9. Mailing address for inventors

Department of Chemical Engineering
University of Texas
Austin, TX 78712

Jeffrey A. Hubbell
3006 Beverly Road
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454-0264

Chandrashekhar P. Pathak
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2501 Lake Austin Blvd
Austin, TX 78712
482-0292

Amarpreet S. Sawhney
1910 Willow Creek, Number 206
Austin, TX 78741
462-1791

10. Signatures of inventors and date

a. Jeffrey A. Hubbell _____ Date [redacted]

b. Chandrashekhar P. Pathak _____ Date

c. Amarpreet S. Sawhney _____ Date

Appendix. Photopolymerization of PEG Gels.

Visible light can be used to polymerize a reaction mixture of monomers when a suitable dye is used as a photoinitiator. For example, the red dye ethyl eosin (maximal absorption at 547 nm) can be used as a photoinitiator with the complementary green light of an Argon-ion laser (514 nm). The chemistry of dye-sensitized photopolymerization is as follows: the laser light excites the dye to a triplit state, whereupon it serves as an electron acceptor. An electron donor is used which easily forms free radicals, such as triethanolamine; a free radical is thus formed. This free radical serves to initiate the free radical polymerization of the double bonds in the reaction mixture. The generation of new free radicals is terminated when the laser light is removed. PEG does not have any double bonds and will not polymerize under these conditions. However, PEG has one hydroxyl group at each end of its polymer chain backbone, which can be easily modified to incorporate double bonds at both end positions. This can be done, for example, by reacting HO-PEG-OH (PEG, showing explicitly the end hydroxyls) with acrylolyl chloride to obtain acrylate-PEG-acrylate, or PEG-diacrylate, which contains one double bond at each end of the PEG polymer chain backbone. In the case of this polymerization, the PEG monomer is itself a polymer; as such it is sometimes referred to as a macromonomer or a prepolymer: the liquid PEG-diacrylate solution in water forms a solid gel with high water content when exposed to the laser light with the photoinitiating dye. PEG-diacrylate radiation polymerization for microbial encapsulation has been addressed by others but photopolymerized PEG-diacrylate has not been used for microbial encapsulation, and polymerized PEG-diacrylate has not been used for biomedical applications such as eukaryotic cell or islet encapsulation.

This photopolymerization system forms a good system for cell encapsulation because (1) it can be performed with visible light, which will have minimal damaging effects on the cells; (2) it can be preformed in the presence of water and oxygen, thus allowing cell viability; and (3) the polymerization reactions are extremely fast (1 - 1000 ms), thus minimizing any toxicity associated with the monomer PEG-diacrylate. The toxicity of the PEG monomers is quite low, since they are actually macromonomers or prepolymers (i.e. large species that cannot cross the cell membrane), and the toxicity of the ethyl eosin photoinitiator and the triethanolamine electron donor is quite low.

The polymerization of PEG-diacrylate is illustrated in Scheme I below, where the PEG polymer backbone is shown as ≈≈≈≈≈≈≈; the strucutre of PEG itself is $HO\{CH_2CH_2O\}_nH$, i.e. each ≈ represents a CH_2CH_2O unit.

Scheme I

$H_2C{=}CHC({=}O)$≈≈≈≈≈≈≈≈≈≈≈≈≈≈≈$C({=}O)CH{=}CH_2$

+

$H_2C{=}CHC({=}O)$≈≈≈≈≈≈≈≈≈≈≈≈≈≈≈$C({=}O)CH{=}CH_2$

514 nm light ↓ with ethyl eosin and triethanolamine

$H_2C{=}CHC({=}O)$≈≈≈≈≈≈≈≈≈≈≈≈≈≈≈≈≈$C({=}O)CH{-}CH_2$

|

$H_2C{-}CHC({=}O)$≈≈≈≈≈≈≈≈≈≈≈≈≈≈≈$C({=}O)CH{=}CH_2$

The laser light converts the PEG macromonomer solution into a gelled three-dimensional network. The properties of this network can be controlled by adjusting the molecular weight of the PEG macromonomer, with smaller macromonomers giving more dense, stiff, and less permeable gels.

The PEG-diacrylate macromonomer will be synthesized from PEG according to Scheme II, as shown below. This is done separately from the gelling reaction in Scheme I and is not done in the

presence of cells. PEG is modified in an anhydrous organic solvent by acryloyl chloride; the acid chloride on the latter reacts rapidly with the hydroxyl end groups on the native PEG molecule.

Scheme II

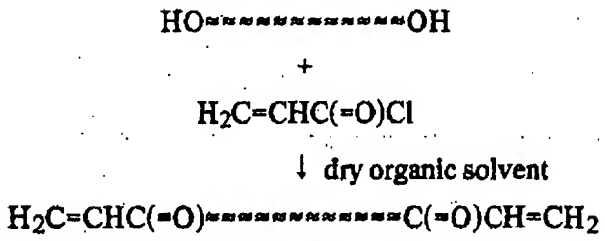

EXHIBIT C

COLLEGE OF ENGINEERING

THE UNIVERSITY OF TEXAS AT AUSTIN

Department of Chemical Engineering · Austin, Texas 78712-1062 · (512) 471-5238 · FAX (512) 471-7060

(512) 471-1690
FAX (512) 471-8799

[redacted]

Patrick Soon-Shiong, M.D.
12303 Dorothy Street
Los Angeles, CA 90049

Dear Patrick:

Enclosed please find three disclosures regarding our recent work in polyethylene glycol gels, entitled "PEO gels for the encapsulation of biological materials," "Visible laser polymerized poly(ethylene glycol) gel coatings for enhanced biocompatibility," and "Biodegradable, cell nonadhesive hydrogels from water soluble, photopolymerizable precursors." Please consider these three disclosures to be confidential. They are communicated to you to facilitate discussion regarding licensing the technologies, and this communication is with the permission of the University.

[redacted]

Sincerely yours,

Jeffrey A. Hubbell
Assistant Professor and
Frank A. Liddell, Jr. Centennial Fellow

EXHIBIT D

PEO GELS FOR THE ENCAPSULATION OF BIOLOGICAL MATERIALS

BY

Chandrashekhar P. Pathak, Amarpreet S. Sawhney and Jeffrey A Hubbell
Department of Chemical Engineering , University of Texas at Austin , Austin TX-78712

Microencapsulation technology holds lot of promise in many areas of medicine. Some important application applications include treatment of diabetes[1], production of biologically important chemicals[2], evaluation of drugs for Anti-Human Immunodeficiency virus[3]. During encapsulation cells are often exposed to processing conditions which are potentially cytotoxic such as heat, organic solvents or changes from physiological pH. These stimuli can often kill cells or affect them in more subtle ways by making them functionally impaired. Even if the cells survive processing conditions, the stringent requirements of encapsulating polymers for biocompatibility, chemical stability, immunoprotection and resistance to cellular growth have made the task even more challenging. The encapsulating method based on ionic crosslinking of alginate[1a] offers relatively mild encapsulating conditions but the long term mechanical and chemical stability of ionically crosslinked polymers remains doubtful. Moreover these polymers are susceptible to cellular growth on implantation *in vivo* [3] which restricts the permeability of the microcapsule to nutrients, metabolites, and transport proteins from the surroundings. This has been seen by Sun et al. to possibly lead to starvation and death of encapsulated islets[4a]. There is a need for a relatively mild encapsulation method which offers control over the properties of encapsulating polymer for this challenging application.

We now report an encapsulation method which we believe is extremely mild, fast and offers a wide control over the properties of the encapsulating polymer. There has been some interest in the use of lasers to initiate free radical polymerization processes[5]. This has resulted into new applications such as stereolithography[6]. These polymerizations are extremely rapid and can be completed in fractions of seconds[6-8]. To demonstrate the ability of these polymerization in rapid gelation of multifunctional acrylic monomers, a plot of the length of the

spike of gel formed by the penetration of the laser beam into the monomer mixture versus laser irradiation time is shown in figure 1. As can be seen from this figure, it takes about 100 ms or less to form a 300 μm diameter gel (a typical size of gel used in microencapsulation technology). This rapid gelation, if used in conjunction with proper choice of monomers can lead to entrapment of cells in a three dimensional covalently bonded polymeric network. The monochromatic laser light will not be absorbed by the cells unless proper chromophore is present and is considered to be harmless if wavelength is more than 434 nm[9,10].

Example 1

Microencapsulation of Animal Cells

Polyethyleneglycol (PEG) diacrylates of different molecular weight were synthesized by a reaction of acryloyl chloride with PEG. A 20 to 30% solution of monomer was mixed with a cell suspension and an initiating system before exposing it through an apparatus shown in figure 2. The droplets from the atomizer were exposed to laser light for about 0.5 to 2 sec to polymerize and make them insoluble in water. The microspheres formed were thoroughly washed with PBS buffer to remove unreacted monomer and residual initiator. Microspheres prepared using this method are shown in figure 3. The size and shape of microspheres was dependent on extrusion rate, atomizer air flow rate and extruding needle diameter. The polymerization times were dependent on initiator concentration, laser power and monomer concentration[7,8]. The polymerizations were carried out at physiological pH in presence of air. This is significant since radical polymerizations may be affected by the presence of oxygen. The process also works at lower temperatures. Cell viability subsequent to encapsulation was checked by trypan blue exclusion assay. Human foreskin fibroblasts (HFF), chinese hamster ovary cells (CHO-K1), and a beta cell insuloma line (RiN5F) were found to be viable (more than 95%) after encapsulation.

Example 2

Biocompatibility of PEO Gels

HFF cells were seeded on these gels at a density of in Dulbecco's modification of eagle's medium containing 10% fetal calf serum. The gels were then incubated at 37°C in a 5% CO2 environment for 4 hr. At the end of this the gels were washed with PBS to remove any non-adherent cells and were observed under a phase contrast microscope at a magnification of 200X. Fig 4 shows the growth of these cells on a typical PEG gel as compared to glass surface. The number of attached cells per field was found to be 3 ± 1 on the gel surfaces as compared to 78 ± 23 for a control glass surface. The cells on these gels appeared rounded and were not in their normal spread morphology, strongly indicating that these gels do not encourage cell attachment.

The in vivo evaluation of extent of inflammatory response to microspheres was carried out by implanting in the peritoneal cavity of mice. Approximately 0.5 ml of microspheres were suspended in 5 ml of sterile HEPES buffered saline. 2.5 ml of this suspension was injected into the peritoneal cavity of ICR male swiss white mice. The microspheres were recovered after 4 days by conducting a lavage of the peritoneal cavity with 5 ml of 10U/ml heparin containing PBS. The extent of cellular growth on the microspheres was visually inspected under a phase contrast microscope. The number of unattached cells present in the recovered lavage fluid were counted using a coulter counter. Fig 5 shows a photograph of microspheres explanted after 4 days showing no or very little coverage. The resistance PEG chain for protein adsorption and hence cellular growth is well documented[11]. The number of unattached cells present in the recovered peritoneal lavage fluid can be considered as an index of irritation and severity of the inflammatory reaction of the host animal to the gel. An increase in cell count is the result of activation of resident macrophages and secretion of chemoattractants and cellular proliferation factors such as interleukins and cytokines which induce the migration of non resident macrophages, neutrophils, lymphocytes to the implant site. They also attract fibroblasts which are responsible for collagen synthesis. These cell counts did not change significantly as

compared to those of a saline vehicle control indicating that these materials do not cause significant irritation of the peritoneal cavity.

Example 4

Permeability of PEO Gels

20 mg of bovine serum albumin was dissolved in 2 ml of a 30% w/v solution of oligomeric PEO 18.5k diacrylate in PBS. This solution was laser polymerized to produce a gel 2cm X 2cm X 0.5 cm in size. The diffusion of bovine serum albumin, human IgG and human fibrinogen (mol wt. 66 kDa, 150 kDa and 350 kDa respectively) was monitored through the 2cm X 2cm face of these gels using a total protein assay reagent (Biorad). A typical release profile for a PEO 18.5K gel is shown in figure 6. This gel allowed a slow transport of albumin but did not allow IgG and fibrinogen to diffuse. This indicates that these gels are capable of being used as immunoprotective barriers. This is a vital requirement for a successful animal tissue microencapsulation material. The release profile was found to be a function of crosslink density and molecular weight of the polyethylene glycol segment of the monomer. The release of any substance from these gels will depend on the crosslink density of the network and and will also depend on the motility of the PEG segments in the network. In the case of short PEO chains between crosslinks, the "pore" produced in the network will have relatively rigid boundaries and so a macromolecule attempting to diffuse through this gel will be predominantly restricted by a sieving kind of effect. If the chain length between crosslinks is long, the chain can fold and move around with a high motility and besides the sieving effect, a diffusing macromolecule will also encounter a free volume exclusion effect. Due to these two contrasting effects a straightforward relation between molecular weight cutoff for diffusion and the molecular weight of the starting oligomer probably does not exist. Yet, it is possible to engineer a desired release profile for a particular protein or a peptide drug by adjusting the crosslink density and length of PEG segments. The 3 dimensional crosslinked covalently bonded polymeric network is expected to be chemically stable for long-term *in vivo* applications.

Example 5

Encapsulation of islets of Langerhans

Islets of langerhans isolated from a human pancreas were obtained as a gift from Transcel Inc. 500 islets suspended in RPMI 1640 medium containing 10% fetal bovine serum were pelleted by centrifuging at 200g for 5 min. The pellet was resuspended in 1 ml of a 30% w/v solution of PEO 18.5K diacrylate in HEPES buffered saline. 5 μl of an ethyl eosin solution in vinyl pyrrolidone (5 mg/ml) was added to this solution along with 100 μl of a 5 M solution of triethanolamine in saline. 20 ml of a mineral oil was then added to the tube which was then vigorously agitated to form a dispersion of droplets 200-500 μm in size. This dispersion was then exposed to an argon ion laser with a power of 250 mW, emitting at 514 nm, for 30 sec. The mineral oil was then separated by allowing the microspheres to settle and the resulting microspheres were washed twice with PBS, once with hexane and finally thrice with media. The viability of the islets was verified by an acridine orange and propidium iodide staining method and also by a trypan blue exclusion test. In order to test functional normalcy a static glucose challenge test was performed on these islets.

Example 6 (Prophetic)

Encapsulation of Neurotransmitter-Releasing Cells

Paralysis agitans, more commonly called Parkinson's disease is characterized by a lack of the neurotransmitter dopamine within the striatum of the brain. Dopamine secreting cells such as cells from the ventral mesencePhalon, from neuroblastoid cell lines or from the adrenal medulla can be encapsulated in a manner similar to that of other cell mentioned in example 1. Cells secreting a precursor for a neurotransmitter, an agonist, a derivative or a mimic of a particular neurotransmitter or cells that have been genetically engineered to be capable of secreting these neurotransmitters, their precursors, derivatives or analogs could also conceivably be encapsulated.

Example 7 (Prophetic)

Encapsulation of Hemoglobin for Synthetic Erythrocytes

Hemoglobin in its free form can be encapsulated in these PEO gels and be slowly released in vivo by diffusion. The diffusion of hemoglobin from the gels can be stopped by the use of polyhemoglobin, which is a crosslinked form of hemoglobin. The polyhemoglobin molecule is too large to diffuse from the PEO gel and encapsulation of this crosslinked hemoglobin could be used to manufacture synthetic erythrocytes. The entrapment of hemoglobin would lead to enhanced circulation times and better stability than that of injection of free hemoglobin.

Example 7 (Prophetic)

Entrapment of Enzymes for Correction of Metabolic Disorders and Chemotherapy

Congenital deficiency of the enzyme catalase causes acatalasemia in mice. Immobilization of catalase in PEO gel networks could provide a way for enzyme replacement. Entrapment of β-glucosidase can similarly be useful in targeting Gaucher's disease. Microspherical PEO gels entrapping urease can be used in extracorporeal blood to convert urea into ammonia. Enzymes such as asparaginase can degrade essential amino acids needed by tumor cells. Immunogenicity of these enzymes prevents the use of these enzymes for chemotherapy. Entrapment of such enzymes in immunoprotective PEO gels could enable successful chemotherapy.

Example 8 (Prophetic)

Cellular Microencapsulation for Evaluation of Anti-Human Immunodeficiency Virus Drugs In Vivo

HIV infected or uninfected human T-lymphoblastoid cells could be encapsulated into PEO gels as described in example 1. These microcapsules could then be implanted in nude mice. The animals could then be treated with the test drugs. After treatment the microcapsules could be harvested and the encapsulated cells screened for viability and metabolic functional normalcy. The survival of infected T cells would indicate successful action of the drug.

CONCLUSION : A new process for cell encapsulation which utilizes the speed of fast visible laser light polymerizations has been successfully employed for cell encapsulation. The rapid polymerization lead to minimum contact with monomers, initiating chemicals and laser irradiation. The properties of the resulting polymers can be tailored for a specific application by choosing appropriate monomers and reaction conditions. The polyethylene glycol based monomers are water soluble, relatively nontoxic and are particularly suitable for this process and the resulting gels are highly biocompatible and resistant to cellular growth.

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FIGURE CAPTIONS

Figure 1 Variation of gel size produced by various laser irradiation times; polymerization of trimethalolpropane triacrylate initiated by triethanol amine (0.1M), ethyl eosin (0.5 mM), argon ion laser emitting at 514nm (power $3.05.10^5 W/m^2$), beam diameter 1 mm, average gel diameter produced 1 mm.

Figure 2 A schematic representation of apparatus used for microencapsulation using laser polymerization

Figure 3 Microspheres produced by laser polymerization of PEG 18.5K diacrylate.

Figure 4 Microspheres recovered from peritoneal cavity of mice after 4 days;

a) alginate-polylysine-alginate microspheres b) PEG (18.5k) microspheres produced by laser polymerization

Figure 5 Human foreskin fibroblasts cultured for 6h on a) Glass coverslip coated with PEG (18.5) gel b) glass coverslip.

Figure 6 The diffusion of albumin, IgG and fibrinogen through PEG (18.5k) gel.